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DATA EVALUATION RECORD[1]

STUDY TYPE: Acute Neurotoxicity - Rats OPPTS 870.6200a [§81-8]; OECD 424.

PC CODE: 016331 **DP BARCODE:** D410187

TEST MATERIAL (PURITY): Momfluorothrin, Lot No.: 9CM0109G (95.7% a.i.)

SYNONYMS: S-1563

CITATION: Shutoh, Y. (2012) S-1563: Acute Oral Neurotoxicity Study in Rats. The Institute of Environmental Toxicology. Ibaraki, Japan. Laboratory Project ID #IE 11-0062, September 19, 2012. MRID 49020036. Unpublished.

SPONSOR: Sumitomo Chemical Co., Ltd. Tokyo, Japan.

EXECUTIVE SUMMARY:

In an acute neurotoxicity study (MRID 49020036), groups of Sprague Dawley rats, (10/sex/dose) were given a single oral dose of S-1563, Lot No.: 9CM0109G (95.7% a.i.) in corn oil by gavage at doses of 0, 30, 80, or 200 mg/kg bw and observed for 14 days. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in all animals at week -1, time of peak effect (6-hours), 7 days and 14 days following administration. At study termination, 5 animals/sex/group were euthanized and perfused in situ for neuropathological examination. Of the perfused animals, 5/sex in the control and high-dose groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

No treatment-related effects were identified on body weight or body weight gain, sensorimotor activity, grip strength, landing footsplay, motor activity, gross or neuropathology.

In the 200 mg/kg group, tremors and slight to moderate salivation were significantly increased in 3 females and 3 males at 6 hours after administration, respectively. In addition, straub tail was observed in 1 male and 1 female of the 200 mg/kg group. One female in the 200 mg/kg group was found dead one day after administration. No abnormalities were identified on necropsy. No further deaths occurred during the observation period. No treatment- related effects were observed in the 30 or 80 mg/kg groups. It is noted that the 10 ml volume of corn oil used as a

1 Disclaimer: The attached Data Evaluation Record is a modified version of the Tier II Summary provided by Sumitomo Chemical Co. Ltd. Portions of this document may have been altered by the EPA reviewer.

vehicle may result in decreased neurotoxic potency of Type-1 pyrethroids (Wolansky 2007).

The LOAEL is 200 mg/kg/day based on increases in tremors and salivation in both sexes at 6 hours after administration. The NOAEL is 80 mg/kg/day.

This neurotoxicity study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424). The lack of stability data was noted as a minor deficiency. However, this is not expected to impact the results of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:	Momfluorothrin
Description:	Yellowish powder
Lot/Batch:	Lot No.: 9CM0109G
Purity:	95.7% (analytically determined)
CAS#:	609346-29-4
Stability:	Expiry date 13 Jan 2013 (after completion of treatment)
2. Vehicle:	Corn oil
3. Test animals:	
Species:	Rat
Strain:	Crl:CD(SD)
Age:	6 weeks (at dosing)
Weight at dosing:	Males 206 to 248 g, females 156 to 197 g
Source:	Charles River, Shiga, Japan Inc
Acclimation period:	14 – 15 days
Diet:	pulverized MF Mash (Oriental Yeast Co., Ltd., Tokyo, Japan)
Water:	Tap water analyzed for contaminants
Housing:	Before grouping 2-5 per cage by sex. After grouping animals were housed individually in clean, stainless steel, wire-mesh cages.
Environmental conditions:	
Temperature:	21.6 - 22.7°C
Humidity:	47.6 - 61.8%
Air changes:	>10 replacements per hour
Photoperiod:	12 hour light / 12 hour dark

B. <u>STUDY DESIGN</u>:

1. In life dates: 28 February – 29 June 2012

2. Animal assignment and treatment

Approximately 6 week-old animals were assigned to groups using a computer generated randomization program. Each group (Groups 1-4) consisted of 10 males and 10 females. The dosing volume was set at a constant volume of 10 mL/kg. Treatment groups received one dose of 30, 80 or 200 mg/kg bw (without overnight fasting) by oral gavage, and controls received vehicle only (corn oil, 10 mL/kg bw). All animals were observed twice daily for mortality and morbidity. Individual body weights were recorded at least weekly, beginning on the day of allocation to groups prior to test compound administration.

TABLE 1. Study design

Experimental parameter	Dose group (mg/kg bw)			
	Control	30	80	200
Total number of animals/sex/group	10	10	10	10
Behavioral testing (FOB, Motor Activity)	10/sex	10/sex	10/sex	10/sex
Neuropathology	5/sex	-	-	5/sex

3. **Test Substance preparation and analysis:** The test substance was suspended in corn oil at a dosing volume of 10 mL/kg bw. Analysis for concentration and homogeneity were performed on each solution before administration. Dosing solutions were sealed in plastic cups and stored in the dark at 3.8 to 5.9ºC until use. Dosing solutions were used within a period of time which stability had been tested (15 days in a dark cold room and 6 hours at room temperature).

Results:
Homogeneity analysis (%RSD): 0.1 to 0.5%

Stability analysis: Reported as stable for 15 days in dark cold room and 6 hours at room temp. Data was not provided.

Concentration analysis: (% nominal): 99 to 105%

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. **Statistics:** Statistical significance of the difference between the control and treated groups was estimated at 5% and 1% levels of probability. The data on activity (in detailed clinical observation), motor activity, grip strength, body weight, body temperature, and landing foot splay were first evaluated by Bartlett's test for equality of variance. When this test showed that variances were homogeneous among groups ($p>0.05$), a parametric one-way analysis of variance (ANOVA) was made to determine if any statistical difference existed among groups. When the result of the analysis of variance was significant ($p\leq0.05$), Dunnett's multiple comparison was applied. When Bartlett's test showed that variances were heterogeneous among groups ($p\leq0.05$), the data was evaluated by Kruskal-Wallis non-parametric analysis of variance. When the result was significant ($p\leq0.05$), a Dunnett type non-parametric multiple comparison was applied. The score data in the detailed clinical observations and functional tests were first evaluated by Kruskal-Wallis non-parametric analysis. When the analysis of variance was significant ($p\leq0.05$), Dunnett type non-parametric multiple comparison was applied. However, statistical evaluation was not performed as to the data of defecation and urination in the detailed clinical observation. Fisher's exact probability (one-tail analysis) was used to analyze the data of mortality rates, and incidences of general health condition. Fisher's Exact probability (two-tail analysis) was used to analyze the data of incidences of non-graded histopathological findings (total incidence). Wilcoxon's sum of ranks test (two-tail analysis) was used to analyze the data of incidences of graded histopathological findings. Statistical evaluation was not performed on

gross pathological findings because there were no abnormalities detected.

C. METHODS / OBSERVATIONS:

1. **Mortality and clinical observations:** Animals were observed at least twice daily for mortality and morbidity. Detailed clinical observations were performed on all surviving animals once prior to the administration (-1 week) and at 6 hours (estimated time of peak effect from DRF), 7 days, and 14 days after administration. Animals were randomized and observed blind to treatment. Observations were performed in and outside the home cage.

2. **Body weight:** Animals were weighed on the day of grouping, at FOB prior to administration, at administration, and 7 days, and 14 days following administration. Mean body weights were calculated for each group. Final body weights were reported on an individual animal basis only.

3. **Food consumption:** Food consumption data was not evaluated.

4. **Cholinesterase determination:** Cholinesterase activity was not determined.

5. **Neurobehavioral assessment:**

   a. **Functional Observational Battery (FOB):** Functional tests were performed on all surviving animals once prior to the administration (-1 week) and at 6 hours, 7 days, and 14 days after the administration in conjunction with the detailed clinical observations. The following parameters were examined: Sensorimotor responses (approach response, touch response, pain response, auditory response, and aerial righting reflex), body temperature, grip strength, landing foot splay, motor activity.

The CHECKED (X) parameters were examined.

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*		Mobility
	Biting	X	Lacrimation* / chromodacryorrhea	X	Rearing+
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
X	Tremors*	X	Piloerection*	X	Convulsions*
X	Abnormal Movements*	X	Fur appearance	X	Tremors*
X	Palpebral closure*	X	Palpebral closure*	X	Abnormal movements*
	Faeces consistency	X	Respiratory rate+	X	Urination / defecation*
		X	Red/crusty deposits*	X	Grooming
	SENSORY OBSERVATIONS	X	Mucous membranes /eye /skin colour	X	Gait abnormalities / posture*
X	Approach response+		Eye prominence*	X	Gait score*
X	Touch response+	X	Muscle tone*	X	Bizarre / stereotypic behaviour*
X	Startle response*				Backing
X	Pain response*		**PHYSIOLOGICAL OBSERVATIONS**		Time to first step
X	Pupil response*	X	Body weight*		
	Eyeblink response	X	Body temperature+		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension				Hindlimb extensor strength
	Hindlimb extension		**OTHER OBSERVATIONS**	X	Forelimb grip strength*
X	Air righting reflex+			X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*
					Rotarod performance

*Required parameters; +Recommended parameters

b. <u>Locomotor activity</u>: Locomotor Activity was evaluated for one hour, in 10-minute intervals, using an automated activity recording system once prior to administration, and at 6 hours, 7 days and 14 days after the administration.

6. <u>Sacrifice and pathology</u>: At termination, necropsy was performed on 5 rats/sex/group, selecting those animals showing the most pronounced findings during the in- life phase. Rats not selected were euthanized and discarded.

The thoracic cavity of animals was opened under anesthesia. The body of each animal was perfused with heparinized 0.1M phosphate buffer followed by a phosphate-buffered 1% glutaraldehyde and 2% paraformaldehyde solution.

The sciatic and tibial nerves of the right side were immersed in the same fixative as used for perfusion. The rest of the tissues were immersed in 10% neutral-buffered formalin.

The CHECKED (X) tissues were evaluated.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain		Mid-thigh
X	Center of cerebrum	X	Sciatic Notch
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons		Sural Nerve
X	Medulla oblongata	X	Tibial Nerve
	SPINAL CORD		Peroneal Nerve
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Lumbar swelling	X	Lumbar dorsal root fibers
	Thoracic swelling	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
	Gasserian Ganglion	X	Cervical dorsal root fibers
	Trigeminal nerves	X	Cervical ventral root fibers
X	Optic nerve		
X	Eyes		
X	Gastrocnemius muscle		

7. **Positive controls:** One study with laboratory project study ID number: RWT-0069, dated 10/09/2009 (page 548) was performed by IET to generate positive control data and validate the procedures and inter-observer reliability, to demonstrate the ability of the performing lab to conduct the FOB and to assess motor activity and neurotoxicity.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs:** In the 200 mg/kg group, tremors and slight to moderate salivation were significantly increased in 3 females and 3 males at 6 hours after administration, respectively. In addition, straub tail was observed in 1 male and 1 female of the 200 mg/kg group. No treatment- related effects were observed in the 30 or 80 mg/kg groups.

TABLE 2. Clinical observations

Observation	Dose Level (mg/kg bw/day)			
	0	30	80	200
Males				
Salivation	0	0	0	3
Females				
Tremors	0	0	0	3

Data were extracted from pages 35-100 of MRID 49020036.
Numbers represent the total number of animals with at least one instance of the observation
n=10

2. Mortality: One female in the 200 mg/kg group was found dead one day after administration. No abnormalities were identified on necropsy. No further deaths occurred during the observation period.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

There were no treatment-related changes in body weights for any dose group of either sex during the 14-day observation period after administration.

C. FOOD CONSUMPTION:

Food consumption was not measured.

D. CHOLINESTERASE ACTIVITIES:

Cholinesterase activity was not measured.

E. NEUROBEHAVIORAL RESULTS:

1. FOB findings:

No treatment-related findings were identified on sensorimotor responses, grip strength, or landing footsplay.

2. Motor activity: No treatment related effects were identified on locomotor activity at any dose level. In the 30 mg/kg groups, females showed significant decreases in motor activity when compared with the control group at one of six 10-minute intervals during the 1 week measurement. However, no significant changes were identified at any other interval, and no dose-response was observed. Therefore, this finding was not considered to be treatment related.

TABLE 3. Motor activity (total activity counts for session)

Test day	Dose level (mg/kg bw)			
	Control	30	80	200
Males				
Week -1	1239 ± 592	1063 ± 612	1105 ± 429	977 ± 370
Week 0	1077 ± 481	1253 ± 381	1689 ± 1328	1455 ± 785
Week 1	2195 ± 404	1902 ± 841	1999 ± 721	1769 ± 722
Week 2	2022 ± 769	1862 ± 483	2223 ± 969	1900 ± 632
Females				
Week -1	1681 ± 734	1705 ± 868	2420 ± 802	1314 ± 529
Week 0	1619 ± 931	2184 ± 1163	2254 ± 861	1273 ± 645
Week 1	2420 ± 1110	1845 ± 1016	1840 ± 450	1489 ± 574
Week 2	1805 ± 814	2070 ± 1135	2198 ± 568	2000 ± 789

Data were extracted from pages 112, 113, 124 and 125 of the study.
Values represent mean ±s.d.
N=10
*=p<.05,** p<.01 compared with controls

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** No macroscopic anomalies were identified in either sex at any dose level.

2. **Brain weight:** Brain weights were not measured.

3. **Neuropathology:** In the 200 mg/kg group, minimal axonal degeneration was found at the cervical swelling (1/5 males and 1/5 females) and lumber swelling (1/5 females) of the spinal cord, proximal sciatic nerve (3/5 males and 1/5 females), and proximal (1/5 males) and calf muscle branch (1/5 males) of the tibial nerve. Retinal dysplasia was found in the eye (1/5 males). In the control group, minimal axonal degeneration was found in the trapezoid body of the pons (1/5 males and 1/5 females), proximal sciatic nerve (3/5 males and 1/5 females) and proximal (1/5 males and 1/5 females) and calf muscle branch of the tibial nerve (1/5 males). Histopathological changes in the high-dose group were considered to be spontaneous as similar findings were identified in the control animals.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

Detailed clinical observations revealed a significant increase in incidence of tremors in 3 females and salivation in 3 males of the 200 mg/kg group at 6 hours after administration. In addition, straub tail was observed in 1 male and 1 female of the 200 mg/kg group. These changes were considered to be suggesting neurotoxic effects at 6 hours after administration.

The no-observed-adverse-effect level (NOAEL) of S-1563 in Sprague-Dawley rats was determined to be 80 mg/kg for both sexes under the conditions of the present acute oral neurotoxicity study.

B. REVIEWER COMMENTS:

Administration of S-1563 via oral gavage resulted in significant increases of tremors and salivation in rats at 200 mg/kg. No further treatment-related effects were identified. In a study conducted by EPAs Office of Research and Development it was found that increased volumes of may influence the neurotoxic potential of pyrethroids (Wolansky 2007) [2]. Therefore, it is noted that the 10 ml volume of corn oil used as a vehicle may have resulted in a decreased neurotoxic potency of momfluorothrin in this study.

2 Wolansky, M., K. McDaniel, V. Moser and K. Crofton (2007). "Influence of dosing volume on the neurotoxicity of bifenthrin" Neurotoxicol Teratol 29: 377-384.

C. STUDY DEFICIENCIES: Minor deficiency: Stability data not provided.